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### **(54) Detecting prostate cancer**

(57) Methods and kits for detecting prostate cancer in urine samples include detecting the methylation status of various genes.

**Description****BACKGROUND OF THE INVENTION**

5 [0001] This invention relates to the interrogation of methylated genes in concert with other diagnostic methods and kits for use with these methods.

[0002] In higher order eukaryotes DNA is methylated only at cytosines located 5' to guanosine in the CpG dinucleotide. This modification has important regulatory effects on gene expression, especially when it involves CpG rich areas (CpG islands) located in gene promoter regions. Aberrant methylation of normally unmethylated CpG islands is a frequent 10 event in immortalized and transformed cells and has been associated with transcriptional inactivation of certain tumor suppressor genes or genes otherwise associated with the amelioration of certain human cancers.

15 [0003] A number of potential methylation markers have recently been disclosed. Glutathione S-transferases (GSTs) are exemplary proteins in which the methylation status of the genes that express them can have important prognostic and diagnostic value for prostate cancer. The proteins catalyze intracellular detoxification reactions, including the inactivation of electrophilic carcinogens, by conjugating chemically-reactive electrophiles to glutathione (C. B. Pickett, et al., Annu. Rev. Blobern., 58:743, 1989; B. Coles, et al., CRC Crit. Rev. Biochem. Mol. Biol., 25:47, 1990; T. H. Rushmore, et al., J. Biol. Chem. 268:11475, 1993). Human GSTs, encoded by several different genes at different loci, have been classified into four families referred to as alpha, mu, pi, and theta (B. Mannervik, et al., Biochem. J., 282:305, 1992). Decreased GSTP1 expression resulting from epigenetic changes is often related to prostate and hepatic cancers.

20 [0004] The S100 proteins are calcium-binding proteins that are implicated in, among other things, tumorigenesis. The family includes S100A2, S100A4, S100A5, S100A6, S100A8, S100A9, and S100A11, which have all been shown to bear some relationship to tumor development though precisely what that role is has not been clear. S 100A6 (calcylin) expression appears to fall off in prostate cancer development. S100A2 has been shown to exhibit lessened expression in breast, lung, and prostate cancer as well. This is believed to be due to hypermethylation of the gene promoter but 25 the picture is not clear since hypermethylation is also seen in non-malignant prostate epithelium and BPH.

[0005] Sampling and sample preparation are important factors in epigenetic testing. Every sample source has its issues. Even biopsy samples taken directly from the affected tissue are known to present the possibility of false negative results due to uneven distribution of affected cells. Urine is a desirable sample because it can be obtained less invasively than many other potential samples. The number and concentration of prostate cancer cells shed into urine can be 30 extremely variable depending on a host of factors such as when the urine is collected, whether it is collected pursuant to prostate massage, and the presence and effect of nucleases and reagents and methods for minimizing their effect.

35 [0006] While some have proposed prostate cancer testing on urine samples, actually producing such a test has proven difficult. First, it is presumed that the basis for such a test is the shedding of cancer cells from the tumor or lesion into the urinary system. Little is actually known about this process. It also seems likely that analyte concentrations could vary much more dramatically than in other samples such as tissue biopsy and even serum samples depending on a wide range of physiological and environmental factors such as the degree to which the patient is hydrated. The stability of the analyte in the matrix is also not well understood in light of the presence of nucleases and a wide variety of other substances that can affect nucleic acids. Sample preparation for a number of other urine assays use spun down samples referred to as sediments. Whether this makes sense for methylation markers cannot be supposed a priori.

40 [0007] Preparation of the patient and pretreatment options are also not well understood. Digital rectal examinations (DRE) are standard diagnostic procedures for determining prostate health in which the physician notes anatomical abnormalities. In the past the outcome of the DRE would be used to determine whether a biopsy or other diagnostic or therapeutic procedure would be necessary. Whether and to what extent procedures such as the DRE or related prostate massage causes cells to slough so that they would then be detected in the subsequent diagnostic procedure was unclear.

45 Procedurally, DRE and digital rectal massage and the time in which they are performed can differ greatly further adding to the list of unknowns in this area.

**SUMMARY OF THE INVENTION**

50 [0008] In one aspect of the invention, a method for characterizing prostate cancer in a patient comprises assaying GSTP1 methylation and one or more control genes in urine within three days of its collection. The assay is considered positive for prostate cancer if the degree of methylation of the GSTP1 exceeds a pre-determined value and is considered negative for prostate cancer if the pre-determined value is not exceeded.

[0009] In another aspect of the invention, a method for characterizing prostate cancer in a patient comprises assaying 55 GSTP1 methylation, one or more control genes, and the S100 gene in urine within three days of its collection. A normalized value of GSTP1 is determined by comparison of the GSTP1 methylation assay value to that of the S100 methylation assay value. The assay is considered positive for prostate cancer if the normalized methylation assay value exceeds a pre-determined value and is considered negative for prostate cancer if the pre-determined value is not exceeded.

[0010] In yet another aspect of the invention, a method for characterizing prostate cancer in a patient comprises assaying GSTP1 methylation and one or more control genes conducted as a nested PCR reaction. The assay is considered positive for prostate cancer if the degree of methylation of the GSTP1 exceeds a pre-determined value and is considered negative for prostate cancer if the pre-determined value is not exceeded.

5 [0011] In yet another aspect of the invention, methylation of the following panels of genes is detected:

- 10 a. GSTP1, APC.
- b. GSTP1, APC, S100A2.
- c. GSTP1, RAR $\beta$ 2.
- d. GSTP1, RAR  $\beta$ 2, S100A2.

[0012] The panels may also include control genes.

[0013] In yet another aspect of the invention, methylation status is determined via quantitative real time PCR.

[0014] In yet another aspect, the invention is a kit useful for the detection of a methylated nucleic acid. The kit includes one or more containers; a first container containing a reagent that modifies unmethylated cytosine and a second container containing a reagent that primes amplification of CpG-containing nucleic acid, wherein the reagent distinguishes between modified methylated and nonmethylated nucleic acid. The kit contains instructions to conduct the assay on patients suspected of having prostate cancer.

[0015] In yet another aspect of the invention, the kit includes a reaction vessel having separate components into which primers are initially stored and wherein during use of the kit, the primers are exuded into a reaction chamber according to a set sequence such that methylation status can be properly assessed. The primers can be for conducting nested amplification reactions.

#### DETAILED DESCRIPTION OF THE INVENTION

[0016] The urine-based assay of this invention is preferably conducted on patients who have had a PSA assay with ambiguous or difficult to determine results (most preferably 2.5-4.0 ng/ml). A negative result using the assay of this invention (in the absence of other clinical indicia) can spare the patient of more invasive testing such as with a biopsy procedure. Thus, viewed most inclusively, one method according to the invention involves first conducting a PSA test in a patient and then conducting the assay described more fully below on those patients having a PSA level assayed at 2.5-4.0 ng/ml.

[0017] The assays of the invention detect hypermethylation of nucleic acids that correspond to particular genes whose methylation status correlates with prostate cancer. A nucleic acid corresponds to a gene whose methylation status correlates with prostate cancer when methylation status of such a gene provides information about prostate cancer and the sequence is a coding portion of the gene or its complement, a representative portion of the gene or its complement, a promoter or regulatory sequence for the gene or its complement, a sequence that indicates the presence of the gene or its complement, or the full length sequence of the gene or its complement. Such nucleic acids are referred to as Markers in this specification. Markers correspond to the following genes only: GSTP1 (Seq. ID. No. 17), APC (Promoter= Seq. ID. No. 18, Gene= Seq. ID. No. 19), RAR $\beta$ 2 (Seq. ID No. 20), S100A2 (Seq. ID. No. 21). Other sequences of interest include constitutive genes useful as assay controls such as beta-Actin (Seq. ID. No. 22 and 23) and PTGS2 (Promoter= Seq. ID. No. 24, Gene= Seq. ID. No. 25).

[0018] Assays for detecting hypermethylation include such techniques as MSP and restriction endonuclease analysis. The promoter region is a particularly noteworthy target for detecting such hypermethylation analysis. Sequence analysis of the promoter region of GSTP1 shows that nearly 72% of the nucleotides are CG and about 10% are CpG dinucleotides.

[0019] The invention includes determining the methylation status of certain regions of the Markers in urine or urethral washes and in which the DNA associated with prostate cancer is amplified and detected. Since a decreased level of the protein encoded by the Marker (i.e., less transcription) is often the result of hypermethylation of a particular region such as the promoter, it is desirable to determine whether such regions are hypermethylated. This is seen most demonstrably in the case of the GSTP1 gene and in the panels indicated in the Summary of the Invention. A nucleic acid probe or reporter specific for certain Marker regions is used to detect the presence of methylated regions of the Marker gene. Hypermethylated regions are those that are methylated to a statistically significant greater degree in samples from diseased tissue as compared to normal tissue.

[0020] As noted above, urine is the matrix in which the assays of this invention are conducted. Most preferably, it is collected after prostate massage and stored at 4C until it can be sedimented. It is most preferably spun down within 4 hours.

[0021] Prostatic massage, when conducted in conjunction with the methylation analyses of the invention, is best conducted as follows: the gland is pressed firmly enough to depress the surface from the base to the apex and from the lateral to the median line for each lobe to ensure the release of sufficient number of prostate cells. It is most preferred that this massage procedure is conducted for 20 seconds or less.

[0022] Some of the primers/probes or reporter reagents of the invention are used to detect methylation of expression control sequences of the Marker genes. These are nucleic acid sequences that regulate the transcription and, in some cases, translation of the nucleic acid sequence. Thus, expression control sequences can include sequences involved with promoters, enhancers, transcription terminators, start codons (i.e., ATG), splicing signals for introns, maintenance of the correct reading frame of that gene to permit proper translation of the mRNA, and stop codons.

[0023] The GSTP1 promoter is the most preferred Marker. It is a polynucleotide sequence that can direct transcription of the gene to produce a glutathione-s-transferase protein. The promoter region is located upstream, or 5' to the structural gene. It may include elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the polynucleotide sequence.

[0024] One method of the invention includes contacting a target cell containing a Marker with a reagent that binds to the nucleic acid. The target cell component is a nucleic acid such as DNA extracted from urine by cell lysis and purification (column or solution based) yielding pure DNA that is devoid of proteins. The reagents include components that prime and probe PCR or MSP reactions and detect the target sequence. These reagents can include priming sequences combined with or bonded to their own reporter segments such as those referred to as Scorpion reagents or Scorpion reporters and described in US Patents 6,326,145 and 6,270,967 to Whitcombe et. al. (incorporated herein by reference in their entirety). Though they are not the same, the terms "primers" and "priming sequences" may be used in this specification to refer to molecules or portions of molecules that prime the amplification of nucleic acid sequences.

[0025] One sensitive method of detecting methylation patterns involves combining the use of methylation-sensitive enzymes and the polymerase chain reaction (PCR). After digestion of DNA with the enzyme, PCR will amplify from primers flanking the restriction site only if DNA cleavage was prevented by methylation. The PCR primers of the invention are designed to target the promoter and transcription region that lies approximately between -71 and +59 bp according to genomic positioning number of M24485 (Genbank) from the transcription start site of GSTP1.

[0026] The method of the invention can also include contacting a nucleic acid-containing specimen with an agent that modifies unmethylated cytosine; amplifying the CpG-containing nucleic acid in the specimen by means of CpG-specific oligonucleotide primers; and detecting the methylated nucleic acid. The preferred modification is the conversion of unmethylated cytosines to another nucleotide that will distinguish the unmethylated from the methylated cytosine. Preferably, the agent modifies unmethylated cytosine to uracil and is sodium bisulfite, however, other agents that modify unmethylated cytosine, but not methylated cytosine can also be used. Sodium bisulfite ( $\text{NaHSO}_3$ ) modification is most preferred and reacts readily with the 5,6-double bond of cytosine, but poorly with methylated cytosine. Cytosine reacts with the bisulfite ion to form a sulfonated cytosine reaction intermediate susceptible to deamination, giving rise to a sulfonated uracil. The sulfonate group can be removed under alkaline conditions, resulting in the formation of uracil. Uracil is recognized as a thymine by Taq polymerase and therefore upon PCR, the resultant product contains cytosine only at the position where 5-methylcytosine occurs in the starting template. Scorpion reporters and reagents and other detection systems similarly distinguish modified from unmodified species treated in this manner.

[0027] The primers used in the invention for amplification of a CpG-containing nucleic acid in the specimen, after modification (e.g., with bisulfite), specifically distinguish between untreated DNA, methylated, and non-methylated DNA. In methylation specific PCR (MSPCR), primers or priming sequences for the non-methylated DNA preferably have a T in the 3' CG pair to distinguish it from the C retained in methylated DNA, and the complement is designed for the antisense primer. MSP primers or priming sequences for non-methylated DNA usually contain relatively few Cs or Gs in the sequence since the Cs will be absent in the sense primer and the Gs absent in the antisense primer (C becomes modified to U (uracil) which is amplified as T (thymidine) in the amplification product).

[0028] The primers of the invention are oligonucleotides of sufficient length and appropriate sequence so as to provide specific initiation of polymerization on a significant number of nucleic acids in the polymorphic locus. When exposed to appropriate probes or reporters, the sequences that are amplified reveal methylation status and thus diagnostic information.

[0029] Preferred primers are most preferably eight or more deoxyribonucleotides or ribonucleotides capable of initiating synthesis of a primer extension product, which is substantially complementary to a polymorphic locus strand. Environmental conditions conducive to synthesis include the presence of nucleoside triphosphates and an agent for polymerization, such as DNA polymerase, and a suitable temperature and pH. The priming segment of the primer or priming sequence is preferably single stranded for maximum efficiency in amplification, but may be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent for polymerization. The exact length of primer will depend on factors such as temperature, buffer, cations, and nucleotide composition. The oligonucleotide primers most preferably contain about 12-20 nucleotides although they may contain more or fewer nucleotides, preferably according to well known design guidelines or rules.

[0030] Primers are designed to be substantially complementary to each strand of the genomic locus to be amplified and include the appropriate G or C nucleotides as discussed above.

[0031] This means that the primers must be sufficiently complementary to hybridize with their respective strands under conditions that allow the agent for polymerization to perform. In other words, the primers should have sufficient complementarity with the 5' and 3' flanking sequence(s) to hybridize and permit amplification of the genomic locus.

[0032] The primers are employed in the amplification process. That is, reactions (preferably, an enzymatic chain reaction) that produce greater quantities of target locus relative to the number of reaction steps involved. In a most preferred embodiment, the reaction produces exponentially greater quantities of the target locus. Reactions such as these include the PCR reaction. Typically, one primer is complementary to the negative (-) strand of the locus and the other is complementary to the positive (+) strand. Annealing the primers to denatured nucleic acid followed by extension with an enzyme, such as the large fragment of DNA Polymerase I (Klenow) and nucleotides, results in newly synthesized + and - strands containing the target locus sequence. The product of the chain reaction is a discrete nucleic acid duplex with termini corresponding to the ends of the specific primers employed.

[0033] The primers may be prepared using any suitable method, such as conventional phosphotriester and phosphodiester methods including automated methods. In one such automated embodiment, diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage, et al. (Tetrahedron Letters, 22:1859-1862, 1981). A method for synthesizing oligonucleotides on a modified solid support is described in U.S. Pat. No. 4,458,066. Any nucleic acid specimen taken from urine or urethral wash, in purified or non-purified form, can be utilized as the starting nucleic acid or acids, provided it contains, or is suspected of containing, the specific nucleic acid sequence containing the target locus (e.g., CpG). Thus, the process may employ, for example, DNA or RNA, including messenger RNA. The DNA or RNA may be single stranded or double stranded. In the event that RNA is to be used as a template, enzymes, and/or conditions optimal for reverse transcribing the template to DNA would be utilized. In addition, a DNA-RNA hybrid containing one strand of each may be utilized. A mixture of nucleic acids may also be employed, or the nucleic acids produced in a previous amplification reaction herein, using the same or different primers may be so utilized. The specific nucleic acid sequence to be amplified, i.e., the target locus, may be a fraction of a larger molecule or can be present initially as a discrete molecule so that the specific sequence constitutes the entire nucleic acid.

[0034] If the extracted sample is impure, it may be treated before amplification with an amount of a reagent effective to open the cells, fluids, tissues, or animal cell membranes of the sample, and to expose and/or separate the strand(s) of the nucleic acid(s). This lysing and nucleic acid denaturing step to expose and separate the strands will allow amplification to occur much more readily.

[0035] Where the target nucleic acid sequence of the sample contains two strands, it is necessary to separate the strands of the nucleic acid before it can be used as the template. Strand separation can be effected either as a separate step or simultaneously with the synthesis of the primer extension products. This strand separation can be accomplished using various suitable denaturing conditions, including physical, chemical or enzymatic means. One physical method of separating nucleic acid strands involves heating the nucleic acid until it is denatured. Typical heat denaturation may involve temperatures ranging from about 80 to 105°C for up to 10 minutes. Strand separation may also be induced by an enzyme from the class of enzymes known as helicases or by the enzyme RecA, which has helicase activity, and in the presence of riboATP, is known to denature DNA. Reaction conditions that are suitable for strand separation of nucleic acids using helicases are described by Kuhn Hoffmann-Berling (CSH-Quantitative Biology, 43:63, 1978). Techniques for using RecA are reviewed in C. Radding (Ann. Rev. Genetics, 16:405-437, 1982). Refinements of these techniques are now also well known.

[0036] When complementary strands of nucleic acid or acids are separated, regardless of whether the nucleic acid was originally double or single stranded, the separated strands are ready to be used as a template for the synthesis of additional nucleic acid strands. This synthesis is performed under conditions allowing hybridization of primers to templates to occur. Generally synthesis occurs in a buffered aqueous solution, preferably at a pH of 7-9, most preferably about 8. A molar excess (for genomic nucleic acid, usually about 10<sup>8</sup>:1, primer:template) of the two oligonucleotide primers is preferably added to the buffer containing the separated template strands. The amount of complementary strand may not be known if the process of the invention is used for diagnostic applications, so the amount of primer relative to the amount of complementary strand cannot always be determined with certainty. As a practical matter, however, the amount of primer added will generally be in molar excess over the amount of complementary strand (template) when the sequence to be amplified is contained in a mixture of complicated long-chain nucleic acid strands. A large molar excess is preferred to improve the efficiency of the process.

[0037] The deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and dTTP are added to the synthesis mixture, either separately or together with the primers, in adequate amounts and the resulting solution is heated to about 90-100°C for up to 10 minutes, preferably from 1 to 4 minutes. After this heating period, the solution is allowed to cool to room temperature, which is preferable for the primer hybridization. To the cooled mixture is added an appropriate agent for effecting the primer extension reaction (the "agent for polymerization"), and the reaction is allowed to occur under conditions known in the art. The agent for polymerization may also be added together with the other reagents if it is heat stable. This synthesis (or amplification) reaction may occur at room temperature up to a temperature at which the agent for polymerization no longer functions.

[0038] The agent for polymerization may be any compound or system that will function to accomplish the synthesis of primer extension products, preferably enzymes. Suitable enzymes for this purpose include, for example, E. coli DNA polymerase 1, Klenow fragment of E. coli DNA polymerase I, T4 DNA polymerase, other available DNA polymerases, polymerase mutants, reverse transcriptase, and other enzymes, including heat-stable enzymes (e.g., those enzymes which perform primer extension after being subjected to temperatures sufficiently elevated to cause denaturing). A preferred agent is Taq polymerase. Suitable enzymes will facilitate combination of the nucleotides in the proper manner to form the primer extension products complementary to each locus nucleic acid strand. Generally, the synthesis will be initiated at the 3' end of each primer and proceed in the 5' direction along the template strand, until synthesis terminates, producing molecules of different lengths. There may be agents for polymerization, however, which initiate synthesis at the 5' end and proceed in the other direction, using the same process as described above.

[0039] Most preferably, the method of amplifying is by PCR. Alternative methods of amplification can also be employed as long as the methylated and non-methylated loci amplified by PCR using the primers of the invention is similarly amplified by the alternative means. In one such most preferred embodiment, the assay is conducted as a nested PCR. In nested PCR methods, two or more staged polymerase chain reactions are undertaken. In a first-stage polymerase chain reaction, a pair of outer oligonucleotide primers, consisting of an upper and a lower primer that flank a particular first target nucleotide sequence in the 5' and 3' position, respectively, are used to amplify that first sequence. In subsequent stages, a second set of inner or nested oligonucleotide primers, also consisting of an upper and a lower primer, are used to amplify a smaller second target nucleotide sequence that is contained within the first target nucleotide sequence. The upper and lower inner primers flank the second target nucleotide sequence in the 5' and 3' positions, respectively. Flanking primers are complementary to segments on the 3'-end portions of the double-stranded target nucleotide sequence that is amplified during the PCR process.

[0040] The first nucleotide sequence within the region of the gene targeted for amplification in the first-stage polymerase chain reaction is flanked by an upper primer in the 5' upstream position and a lower primer in the 3' downstream position. The first targeted nucleotide sequence, and hence the amplification product of the first-stage polymerase chain reaction, has a predicted base-pair length, which is determined by the base-pair distance between the 5' upstream and 3' downstream hybridization positions of the upper and lower primers, respectively, of the outer primer pair.

[0041] At the end of the first-stage polymerase chain reaction, an aliquot of the resulting mixture is carried over into a second-stage polymerase chain reaction. This is preferably conducted within a sealed or closed vessel automatically such as with the "SMART CAP" device from Cepheid. In this second-stage reaction, the products of the first-stage reaction are combined with specific inner or nested primers. These inner primers are derived from nucleotide sequences within the first targeted nucleotide sequence and flank a second, smaller targeted nucleotide sequence contained within the first targeted nucleotide sequence. This mixture is subjected to initial denaturation, annealing, and extension steps, followed by thermocycling as before to allow for repeated denaturation, annealing, and extension or replication of the second targeted nucleotide sequence. This second targeted nucleotide sequence is flanked by an upper primer in the 5' upstream position and a lower primer in the 3' downstream position. The second targeted nucleotide sequence, and hence the amplification product of the second-stage PCR, also has a predicted base-pair length, which is determined by the base-pair distance between the 5' upstream and 3' downstream hybridization positions of the upper and lower primers, respectively, of the inner primer pair.

[0042] The amplified products are preferably identified as methylated or non-methylated with a probe or reporter specific to the product as described in US Patent 4,683,195 to Mullis et. al., incorporated herein by reference in its entirety. Advances in the field of probes and reporters for detecting polynucleotides are well known to those skilled in the art. Optionally, the methylation pattern of the nucleic acid can be confirmed by other techniques such as restriction enzyme digestion and Southern blot analysis. Examples of methylation sensitive restriction endonucleases which can be used to detect 5'CpG methylation include SmaI, SacI, EagI, Mspl, HpaII, BstUI and BssHII.

[0043] In another aspect of the invention a methylation ratio is used. This can be done by establishing a ratio between the amount of amplified methylated species of Marker attained and the amount of amplified reference Marker or non-methylated Marker region amplified. This is best done using quantitative real-time PCR. Ratios above an established or predetermined cutoff or threshold are considered hypermethylated and indicative of having a proliferative disorder such as cancer (prostate cancer in the case of GSTP1). Cutoffs are established according to known methods in which such methods are used for at least two sets of samples: those with known diseased conditions and those with known normal conditions. The reference Markers of the invention can also be used as internal controls. The reference Marker is preferably a gene that is constitutively expressed in the cells of the samples such as Beta Actin.

[0044] Established or predetermined values (cutoff or threshold values) are also established and used in methods according to the invention in which a ratio is not used. In this case, the cutoff value is established with respect to the amount or degree of methylation relative to some baseline value such as the amount or degree of methylation in normal samples or in samples in which the cancer is clinically insignificant (is known not to progress to clinically relevant states or is not aggressive). These cutoffs are established according to well-known methods as in the case of their use in methods based on a methylation ratio.

[0045] In the most preferred embodiment of the invention, GSTP1 methylation values obtained by MSP or other suitable methods are normalized with S100A2 methylation values determined using the same method. The normalized value is obtained by subtracting the S100A2 assay value from that of the GSTP1 value as shown in Example 7. Other normalization methods can also be used such as generation of a methylation ratio (obtained by converting the Ct value to a copy number for the gene of interest and dividing that copy number by the copy number for beta-actin, obtained in the same manner). When using a normalized value, the cutoff value is determined by first generating a training set in which the cutoff generates optimal sensitivity and specificity and then validating the cutoff in an independent validation set.

[0046] The inventive methods and kits can include steps and reagents for multiplexing. That is, more than one Marker can be assayed at a time. But only the following Markers are assayed as part of this invention GSTP1, RAR-  $\beta$ 2, APC, and S100A2 along with internal controls such as  $\beta$ -Actin.

[0047] Since a decreased level of transcription of the gene associated with the Marker is often the result of hypermethylation of the polynucleotide sequence and/or particular elements of the expression control sequences (e.g., the promoter sequence), primers prepared to match those sequences were prepared. Accordingly, the invention provides methods of detecting or diagnosing a cell proliferative disorder by detecting methylation of particular areas, preferably, within the expression control or promoter region of the Markers. Probes useful for detecting methylation of these areas are useful in such diagnostic or prognostic methods.

[0048] The kits of the invention can be configured with a variety of components provided that they all contain at least one primer or probe or a detection molecule (e.g., Scorpion reporter). In one embodiment, the kit includes reagents for amplifying and detecting hypermethylated Marker segments. Optionally, the kit includes sample preparation reagents and /or articles (e.g., tubes) to extract nucleic acids from samples.

[0049] In a preferred kit, reagents necessary for one-tube MSP are included such as, a corresponding PCR primer set, a thermostable DNA polymerase, such as Taq polymerase, and a suitable detection reagent(s) such as hydrolysis probe or molecular beacon. In optionally preferred kits, detection reagents are Scorpion reporters or reagents. A single dye primer or a fluorescent dye specific to double-stranded DNA such as ethidium bromide can also be used. The primers are preferably in quantities that yield high concentrations. Additional materials in the kit may include: suitable reaction tubes or vials, a barrier composition, typically a wax bead, optionally including magnesium; necessary buffers and reagents such as dNTPs; control nucleic acid (s) and/or any additional buffers, compounds, co-factors, ionic constituents, proteins and enzymes, polymers, and the like that may be used in MSP reactions. Optionally, the kits include nucleic acid extraction reagents and materials.

## EXAMPLES

### Example 1: Sample Preparation and MSPCR

[0050] Prostate samples were obtained from patients with known clinical outcomes.

[0051] The methylation assays were conducted as follows. Genomic DNA was modified using a commercially available sodium bisulfite conversion reagent kit (Zymo Research, Orange, CA, USA). This treatment converted all Cytosines in unmethylated DNA into Uracil, whereas in methylated DNA only cytosines not preceding guanine were converted into Uracil. All cytosines preceding guanine (in a CpG dinucleotide) remained as cytosine. The assays are described more fully below.

a. Sedimentation: Sedimented urine samples were obtained as follows.

[0052] 50-ml Falcon tubes containing the urine were centrifuged at 3000g on VRX Sorvall centrifuge for 10 minutes at +4 degrees C. Supernatant was removed leaving ~5ml on top of the pellet. The tubes were then spun down again (3000g for 5 minutes) in order to discard the remaining supernatant (using 1 ml tips). Urine sediment was then rinsed with 20 mL cold (4C) PBS, spun down again (3000g for 5 minutes), and the residual supernatant was aspirated. Samples were then stored at -20C.

b. Cell Lysis and DNA extraction:

[0053] The cells in the sediment were then lysed as follows. 700  $\mu$ l Cell Lysis Solution was added to each sample containing a urine cell pellet. The lysate was then transferred in a 2.0 ml microfuge tube and 3  $\mu$ l Proteinase K Solution (20 mg/ml) was added to the lysate, mixed by inverting 25 times, and incubated for one hour to overnight at 55°C.

[0054] Samples were cooled to room temperature by placing at 20°C (heat block) for 10 minutes. 300  $\mu$ l Protein Precipitation Solution was then added to the lysate which was then vortexed vigorously at high speed for 20 seconds. The samples were placed into an ice bath for 5 minutes and centrifuged at (16000 RPM) for 5 minutes. The precipitated proteins formed a tight pellet. The supernatant was then transferred to a new 2.0 ml tube with the precipitation steps

repeated.

[0055] The supernatant containing the DNA was then transferred into a clean 2.0 ml microfuge tube and centrifugation was repeated (16000 RPM for 3 minutes) with the supernatant again transferred into a clean 2.0 ml microfuge tube containing 900  $\mu$ l 100% isopropanol and 2  $\mu$ l Glycogen 20 mg/ml. The sample was mixed by inverting gently 50 times and kept at room temperature for at least 10-15 minutes on the rocker and then cooled to - 20C. The sample was then centrifuge at 16000 RPM for 5 minutes. The DNA was then visible as a small white pellet. Supernatant was removed with the 1ml-pipet and the sample was ccentrifuge at (16000 RPM) for 60 seconds. Remaining supernatant was removed with a 100 $\mu$ l-pipet. 900  $\mu$ l 70% ethanol was added and the tube was inverted 10 times to wash the DNA pellet followed by another centrifugation at 16000 RPM for 1 minute. Ethanol was discarded with the 1ml-pipet followed by another centrifugation at (16000 RPM) for 60 seconds.The remaining supernatant was discarded with the 100 $\mu$ l-pipet and the sample was allowed to air dry 10-15 minutes.

[0056] 45  $\mu$ l LoTE buffer was added to the dried samples and the DNA was rehydrated by incubating at 65°C for 1 hour shaking at 1100 rpm and overnight at 20°C shaking at 1100 rpm. The DNA was stored in a clearly labelled tube at -80°C.

c. Bisulfite modification:

[0057] DNA Samples were then modified using EZ-DNA methylation kit from ZymoResearch (Cat. No D5001) as follows.

[0058] 24 ml absolute Ethanol was added to the M-Wash buffer Concentrate to make the final M-Wash buffer. 5  $\mu$ l of M-Dilution Buffer directly to 45  $\mu$ l of the DNA sample. This mixture was mixed by pipetting up and down and then spun briefly followed by incubation at 37°C for 15 minutes in a heat block with shaking at 1100 rpm. During the incubation, CT Conversion Reagent was prepared by adding 750  $\mu$ l Baker Water and 210  $\mu$ l of M-Dilution Buffer. It was then mixed by vortexing for 1 minute every 2 minutes for a total of 10 minutes. After the above incubation, 100  $\mu$ l of the prepared CT Conversion Reagent (after briefly spinning) was added to each sample which was then vortexed lightly and spun briefly. The sample was then incubated at 70°C for 3 hour with the heating block (shaking at 1100 rpm) covered with aluminum foil.

[0059] The sample was then spun down briefly and set on ice for 10 minutes. 400  $\mu$ l of M-Binding buffer was added to the sample which was mixed by pipetting up and down. All the supernatant was loaded into a Zymo-Spin Column which was placed into a 2 ml collection tube. The tube was centrifuged at maximum speed for 15 - 30 seconds the flow-through discarded. 200  $\mu$ l of M-Wash Buffer was added to the column which was centrifuge at maximum speed for 15 - 30 seconds again with the flow-through again discarded.

[0060] 200  $\mu$ l of M-Desulphonation Buffer was added to the column and let to stand at room temperature for 15 minutes followed by centrifugation at maximum speed for 15 -30 seconds with the flow-through discarded. This procedure was followed three times with the last centrifugation step lasting 30 seconds. The column was then placed onto a clean 1.5 ml tube to which 50  $\mu$ l of M-elution buffer was added. The columns were then let to stand for 1 min at RT followed by centrifugation at maximum speed for 1 minute to elute the DNA. The eluted DNA was labeled and stored as 'BT modified' at -80°C.

[0061] MSPCR assays were then set up with the following primers and probes:

Outer PCR primers

GSTP1_332_U18	Seq ID No. 1	TCGGGGATTTAGGGCGT
GSTP1_513_L21	Seq ID No. 2	ACGAAAACCTACGACGACGAAA
Actin_309_U24	Seq ID No. 5	GATATAAGGTTAGGGATAGGATAG
Actin_501_L22	Seq ID No. 6	AACCAATAAAACCTACTCCTCC
APC_Outer_692_U19	Seq ID No. 9	CCCTATACCCCCTACGAA
APC_Outer_830_L25	Seq ID No. 10	GGCAGGGTTGTATTAATATAGTTATA
RARB2_Outer_16_U25	Seq ID No. 13	GGAAGTGAGTTAGAGGTAGGA
RARB2_Outer_239_L25	Seq ID No. 14	TCCAAACTTACTCGACCAATCCAAC

## Inner PCR Scorpion probe/primer sets

Description	Sequence	Seq ID No
GSTP1 Scorpion	FAM-CGCACGGCGAACTCCCGCCGACGTGCG BHQ-HEG-TGTAGCGGTCGTCGGGGTTG	3
GSTPi Reverse Primer	5' GCCCAATACTAAATCACGACG 3'	4
Actin Scorpion	Q670-CCGCGCATCACCAACCCCACACGCGCGG- BHQ2-HEG-GGAGTATATAGTTGGGAAGTTG	7
Actin Reverse Primer	5' AACACACAATAACAAACACAAATTAC 3'	8
APC Scorpion	Texas Red - GCCGGCGGGTTTCGACGGGCCGGC- BHQ2-HEG-CGAACCAAAACGCTCCCCA	11
APC Lower Primer	GTCGGTTACGTGCGTTATATTAG	12
RARB2 Scorpion	Q570 - CGCGCCCCGACGATACCCAAAGCGCCG- BHQ2-HEG- AACGCGAGCGATTGAGTAG	15
RARB2 Lower Primer	CTTACAAAAAAACCTTCCGAATACG	16

BHQ= Black Hole Quencher reporter molecule. HEG= hexaethylene glycol

[0062] Nested PCR reactions were conducted using "SMARTCAP" tubes (Cepheid) and the "SMARTCYCLER" (Cepheid) PCR analyzer as follows.

[0063] Thawed reagents were each vortexed briefly to mix. Adequate Cepheid SmartCap PCR reaction tubes were labeled and placed in the rack. Using a fresh pipette tip for each tube, 5  $\mu$ l of the first round PCR master mix were added into each tube. Again with a fresh pipette tip for each specimen, 5  $\mu$ L of specimen were added to the respective tubes which were then closed without snapping SmartCaps in place. The tubes were centrifuged for 30 seconds in the "SMARTCYCLER" centrifuge and placed in sequence in the

[0064] "SMARTCYCLER" instrument with the lid on the "SMARTCYCLER" closed instrument and the run initiated run. The cycling conditions on the Cepheid platform are indicated below (1<sup>st</sup> round PCR).

[0065] Following completion of the run, the tubes were removed and a second round of PCR was set up as follows. The tube lid was opened followed by the addition of 15  $\mu$ l of the second round PCR master mix into the "SMARTCAP" reservoir to the final volume of 25  $\mu$ l. The spike was inserted and the lid was snapped into place. The tubes were then centrifuged for 30 seconds in the microcentrifuge with a suitable rotor. The inner PCR reaction was then run for 40 cycles under cycling conditions on the Cepheid platform as indicated below (2nd round PCR). Following completion of the run, the Cepheid tubes were removed and discarded.

[0066] The following cycling parameters were used.

First Round PCR		
Temperature	Time	Cycles
94C	2min	1
92C	20sec	
55C	30sec	18
70C	30sec	
70C	5min	1
Second Round PCR		
Temperature	Time	Cycles
95C	1min	1

(continued)

Second Round PCR		
Temperature	Time	Cycles
95C	20sec	40
59C	30sec	collection

[0067] The reaction mix for a single quadruplex in the SMARTCAP tubes was prepared using the following individual components.

• First round PCR (R1)

[0068]

Master Mix (MM1)	
Reagents	ul
DNA template (ul)	5.00
10x Magic Buffer	1
Taq (Ab) Polymerase	0.5
10x outer Primer Mix	1
2.5 mM dNTPs (100nM)	0.4
Water	2.10
Total	10.0
Outer primer PM-1 final Conc: All /Actin markers-0.05uM -0.04uM	

• Second Round PCR (R2)

[0069]

Master Mix (MM2)	Pre-mix (ul w/o sample)
Reagents	ul
DNA template (ul)	0.0
10x Magic Buffer	1.5
Taq (Ab) Polymerase	1.5
25x inner primer Mix -4p	1
25 mM dNTPs (1mM)	1
Water	10.0
Total	15.0
Inner primer final Conc: GSTP1/RARB/APC- 0.4uM/Actin-0.24uM	

[0070] The PCR Master Mixes were prepared as follows (outer primer and inner Scorpion probe/primer mixes)

5                   **10X outer primer Mix-4p -GSTP1-0.5/RARB-0.5/APC-0.5uM/Actin-0.4uM**

10                   Outer primer final Concentrations: GSTP1-0.05/RARB-0.05/APC-0.05uM/Actin-0.04uM

15                   Calculations are shown for a single reaction and a batch of 200.

Primer concentration	ul per 1 rxn	200
100 uM GSTP1_332_U18	0.005	1
100 uM GSTP1_513_L21	0.005	1
100 uM APC_Outer_692_U19	0.005	1
100 uM APC_Outer_830_L25	0.005	1
100 uM RARB2_Outer_16_U25	0.005	1

100 uM RARB2_Outer_239_L25	0.005	1
100 uM Actin_309_U24	0.004	0.8
100 uM Actin_501_L22	0.004	0.8
Water	0.962	192
Total	1	200

25X inner primer/probe Mix -4p (10uM each/6uM actin)

30                   Inner primer final Concentrations: GSTP1/RARB/APC- 0.4uM/Actin-0.24uM. Calculations are shown for a single reaction and a batch of 200.

Primer concentration	ul per 1 rxn	200
100 uM GSTP1_Fam_Sc_1112_L15	0.1	20
100 uM GSTPi_1151_L22	0.1	20
100 uM RARB2_M_136_AS15_Q570	0.1	20
100 uM RARB2_165_L24	0.1	20
100 uM APC_M_781_AS15_TR	0.1	20
100 uM APC_804_L25	0.1	20
100 uM Actin_Q670_Sc_382_L15 (Cy5)	0.06	12
100 uM Actin_425_L27	0.06	12
Water	0.28	56
Total	1	200

[0071] The final 25  $\mu$ l reaction contents were as follows:

Component	Final Conc in Rxn
$(\text{NH}_4)_2 \text{SO}_4$	16.6mM
Tris pH 8.8	67mM

(continued)

Component	Final Conc in Rxn
MgCl <sub>2</sub>	6.7mM
B-ME	10mM
Each dNTP	1.25mM
Each Primer	400nM
Each Scorpion	400nM
Tris-HCl, pH 8.3	4mM
KCl	20mM
EDTA	0.02mM
DTT	0.2mM
Nonidet P40	0.02% (v/v)
Tween 20	0.02% (v/v)
Glycerol	2% (v/v)
TP6-25 antibody	0.65 ug
Taq Polymerase	5U
Tris-HCl, pH 7.6	1.8mM
Glycerol	0.70%
ProClin 300	0.02%

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[0072] Data output from the "SMARTCYCLER" analyzer.

[0073] Data were analyzed through the implementation of predetermined thresholds and criteria are shown in Table 1.

Table 1

Ch #	Threshold	Valid Min Cycle	Valid Max Cycle	Bkgd Min Cycle	Bkgd Max Cycle	Box Car Avg.
FAM	30	13	40	5	45	0
Cy3	20	13	40	5	45	0
TxR	20	13	40	5	45	0
Cy5	20	13	40	5	45	0

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[0074] Results were generated and are presented as the following assay performance characteristics: % Sensitivity, Specificity and 95%confidence intervals, calculated for the combination of markers at defined Ct cutoffs for 2 in 1 PCR format. Area under the curve values were calculated based on ROC curve analysis performed with two statistical software packages. For a single marker analysis, AUC values were generated using MedCalc software and for different combinations of multiple markers, logistic regression model in S-Plus statistical software was applied.

[0075] A cut-off value was set based on the relative distribution of Ct values between the cancer and non-cancer patients. If either one of Ct values from the set of methylation markers was below the defined cutoff, the sample was considered methylated, even if Actin indicated the "no test" case. The figures show the data compared across a variety of parameters to illustrate the various embodiments of the invention.

#### Example 2: Effect of Sample Storage and Panel Identification

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[0076] The procedure described in example 1 was repeated with combinations assays comprising a combination of Markers from the GSTP1, RAR $\beta$ 2, and APC genes. Assays were conducted within 3 days of sample collection, 5 days of sample collection, and 16 days of sample collection as indicated. Results are shown in Table 2.

Table 2.

GSTP	RAR $\beta$ 2	APC	Days Stored	Sensitivity (%)	Specificity (%)
X			<b>16</b>	31	96
		X	<b>16</b>	31	93
	X		<b>16</b>	36	86
X		X	<b>16</b>	44	91
X	X		<b>16</b>	39	85
X	X	X	<b>16</b>	49	82
X			<b>5</b>	35	96
		X	<b>5</b>	35	91
	X		<b>5</b>	40	85
X		X	<b>5</b>	50	90
X	X		<b>5</b>	44	84
X	X	X	<b>5</b>	54	81
X			<b>3</b>	36	91
		X	<b>3</b>	32	88
	X		<b>3</b>	29	93
X		X	<b>3</b>	54	88
X	X		<b>3</b>	39	84
X	X	X	<b>3</b>	54	81

**[0077]** The sample set for this example were whole (neat) urine samples and consisted of 148 samples (68 known cancers) for the 16 day set, 121 samples (52 known cancers) for the 5 day set, and 73 samples (30 known cancers) for the three day set. Surprisingly, the combination of GSTP and RAR $\beta$ 2 outperformed the combination of GSTP, RAR $\beta$ 2, and APC despite APC being a known prostate cancer marker. This two-gene combination vastly outperformed any other when samples were stored for three days or less. When all tests were considered, the positive predictive value for samples stored for 3 days was 65.9% compared to 51.35 % for samples held for 5 days, and 47.22% for samples stored for 16 days. ROC curves analyses were then conducted for new data sets (N=73, known cancers=30, known non-cancers = 43) using whole urine samples and using sediments prepared as described above and stored for 3 days. The area under the curve was determined for individual Markers. Results are summarized in Table 3

Table 3

Marker	Sample	Approximate Sens/Spec (%)	AUC
GSTP	WU	38/93	.66
RAR $\beta$	WU	31/93	.58
APC	WU	35/98	.64
GSTP	Sediment	43/90	.64
RAR $\beta$	Sediment	56/77	.64
APC	Sediment	52/87	.62

**[0078]** GSTP by itself gave the best results by ROC analysis when samples were whole urine (neat). Spinning the sample down to sediments improved the performance of the RAR $\beta$  Marker tremendously as shown by this same analysis.

**Example 3: Prostate Massage**

[0079] Two additional sample sets were tested and analyzed to determine the effect of prostatic massage on the performance of the urine based assay. In the first sample set, 36 samples (20 known cancers) were obtained from patients having prostatic massage limited to less than 20 seconds. In the other sample set, 77 samples (30 known cancers) were obtained from patients having prostatic massage for more than 20 seconds. In each case, samples were stored for five days or less. The results of the MSPCR conducted on these samples are summarized in Table 4.

Table 4

GSTP	RAR $\beta$ 2	APC	Massage (seconds)	Sensitivity (%)	Specificity (%)
X			<20	39	100
		X	<20	33	93
		X	<20	39	80
		X	<20	50	87
X		X	<20	56	93
X	X		<20	56	87
X	X	X	<20	61	87
X	X	X	<20	61	93
X			>20	33	93
		X	>20	37	91
		X	>20	41	76
		X	>20	37	84 .
X		X	>20	48	89
X	X		>20	41	82
X	X	X	>20	48	82
X	X	X	>20	52	82

[0080] The best results were obtained from the panel that included GSTP, RAR $\beta$ , and APC when the prostate massage was less than 20 seconds in duration. This is surprising as one would have expected lengthier massage to release more cells and increase, at the least, the specificity.

**Example 4. Digital Rectal Examination**

[0081] Four additional sample sets were tested and analyzed to determine the effect of selection on the basis of an abnormal versus a normal digital rectal examination (DRE) on the performance of the urine based assay. In the first sample set, 64 whole urine samples (23 known cancers) were obtained from patients having a normal DRE. In the second sample set 33 whole urine samples (19 known cancers) were obtained from patients having an abnormal DRE. The third sample set contained 48 sedimented samples (21 known cancers) who presented with a normal DRE and the fourth sample set contained 22 sedimented samples (8 known cancers) of from patients with abnormal DREs. In each case, samples were stored for five days or less. The results of the MSPCR conducted on these samples are summarized in Table 5.

Table 5

GSTP	RAR $\beta$ 2	APC	Sample	DRE	Sens(%)	Spec(%)
X			Whole	Negative	20	95
		X	Whole	Negative	30	87
	X		Whole	Negative	25	79

(continued)

	<b>GSTP</b>	<b>RAR<math>\beta</math>2</b>	<b>APC</b>	<b>Sample</b>	<b>DRE</b>	<b>Sens(%)</b>	<b>Spec(%)</b>
5	X		X	<b>Whole</b>	<b>Negative</b>	35	85
	X	X		<b>Whole</b>	<b>Negative</b>	25	82
	X	X	X	<b>Whole</b>	<b>Negative</b>	40	72
10	X	X	X	<b>Whole</b>	<b>Negative</b>	40	74
				<b>Whole</b>	<b>Abnormal</b>	60	92
			X	<b>Whole</b>	<b>Abnormal</b>	60	92
		X		<b>Whole</b>	<b>Abnormal</b>	53	85
15	X		X	<b>Whole</b>	<b>Abnormal</b>	80	92
	X	X		<b>Whole</b>	<b>Abnormal</b>	67	85
	X			<b>Sediment</b>	<b>Negative</b>	19	88
20		X		<b>Sediment</b>	<b>Negative</b>	10	88
		X		<b>Sediment</b>	<b>Negative</b>	48	80
			X	<b>Sediment</b>	<b>Negative</b>	33	76
25	X	X		<b>Sediment</b>	<b>Negative</b>	52	76
	X		X	<b>Sediment</b>	<b>Negative</b>	38	72
	X			<b>Sediment</b>	<b>Abnormal</b>	71	85
30	X			<b>Sediment</b>	<b>Abnormal</b>	86	77
		X		<b>Sediment</b>	<b>Abnormal</b>	57	62
		X		<b>Sediment</b>	<b>Abnormal</b>	57	85
35			X	<b>Sediment</b>	<b>Abnormal</b>	86	92
	X	X		<b>Sediment</b>	<b>Abnormal</b>	71	77
	X		X	<b>Sediment</b>	<b>Abnormal</b>	86	75

**[0082]** Markers used with samples selected from patients with an abnormal DRE performed substantially better than the cases in which patients had negative DREs. Whole urine samples from patients with abnormal DREs were assayed with nearly the same degree of sensitivity and specificity as the best sedimented samples when the Marker panel was made up of GSTP and APC. A single Marker (APC) assay performed the best in sedimented samples with an abnormal DRE but the GSTP/APC panel was not far behind.

#### Example 5: PSA Level

**[0083]** Two additional samples sets were tested and analyzed to determine the effect of sample selection based on PSA level. In the first sample set, -52 whole urine samples ( 25 known cancers) were obtained from patients having a PSA value of 2.5-4 ng/ml. In the other sample set, 169 samples (80 known cancers) were obtained from patients having a PSA level of 4-10 ng/ml. In each case, samples were stored within five days at 4° C between urine collection and sedimentation procedures. The results of the MSPCR conducted on these samples is summarized in Table 6 below.

**[0084]** For 52 subjects with PSA levels between 2.5 and 4 ng/mL (including 25 cancer and 27 non-cancer cases), sensitivity of 58% and specificity of 88% was demonstrated when using logistic regression model, or 58% and 81%, respectively, when using 3 markers with the following Ct cutoffs: GSTP=26, RAR=28, APC=25 and no test rate at 3.8%. For patient co-hort with PSA 4-10, sensitivity/specificity characteristics were 59/65% using the same markers and Ct cutoffs. Two-sample T-test confirmed that there was no statistically significant difference in assay performance between two co-horts with PSA ranges 2.5-4 and 4-10 ng/mL (P=0.000). Results are shown in Table 6.

Table 6

Marker	GST D2	RAR	APC	Sens, %	Spec, %	AUC	No test rate, %	
PSA 2.5-4, n=52, C=25, NC=27	28			38	85	0.619	3.8	
	26			26	96			
		28		54	85	0.712		
			26	25	81	0.574		
	26		28	54	73	0.622		
	Logistic regression model			38	85			
	26	28		54	81	0.697		
	Logistic regression model			58	85			
	26	27	25	50	81			
	26	27	25	58	81	0.688		
	Logistic regression model			58	88			
PSA 4-10, n=169, C=80, NC=89	28			49	90	0.652	5.9	
	26/30			34/44	95/84			
		28/26		48/40	70/84	0.602		
			26	40	83	0.616		
	26		28	62	70	0.634		
	28		27	59	74			
	Logistic regression model			45	85			
	26	28		53	70	0.644		
	Logistic regression model			45	85			
	26	28	25	59	65			
	26	27	25	56	73	0.651		
	Logistic regression model			45	87			

**Example 6: More Extensive Multiplexing**

[0085] More extensive multiplexing was conducted using additional markers known to be useful in prostate cancer analysis. Maximum assay specificity was sought given that the objective of the assay was to resolve ambiguous PSA results (2.5-4 ng/ml in clinical use). The assays were performed on sediment samples stored for 3 days. One round of PCR was conducted on each of 60 samples (30 from cancer cases, 30 from non-cancer samples). Data with urine sediments generated for 6 markers (GST+RAR+APC+RASS+CDH1+PDLIM4) using 3 quadruplex reactions demonstrate that CDH1, RASS and PDLIM4 do not add value to maintain specificity at 80%. Inclusion of all 6 markers impairs assay specificity (sens=44%, spec =63%). Moreover, use of 6 markers does not suit the singletube assay format. Results are shown in Table 7.

Table 7.

GSTP1	RAR	APC	RASSF1A	CDH1D2	PDLIM4D2	Sens	Spec
X	X		X		X	41 %	80%
X	X			X	X	44%	70%
X	X		X		X	37%	83%
X	X				X	41 %	73%
X	X	X	X	X	X	44%	63%

(continued)

GSTP1	RAR	APC	RASSF1A	CDH1D2	PDLM4D2	Sens	Spec
X	X	X				38%	80%

5

**Example 7: Normalization of Results using S100A2**10 [0086] Urine samples were tested using four markers (GSTP1, RAR $\beta$ 2, APC, and S100A2) in MPCR reactions as described above.

Table 7.

Pru-Mu					
			GSTP1	RAR	APC
Sensitivity			20.00%	5.00%	5.00%
Specificity			90.00%	100.00%	90.00%
GSTP1/APC//RAR $\beta$ 2					
Sensitivity				20.00%	
Specificity				80.00%	

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25 [0087] The data above show performance of three markers individually and as a combination on a representative set of 20 Cancers and 10 Non-cancers. The sensitivity is lower than typically observed due to the less than optimal storage time of the urine samples prior to sedimentation.

30 [0088] The same samples were then analyzed with S100 and the delta Ct between S100 and the gene of interest was used to generate a cutoff. The data show improved performance for RAR $\beta$ 2 and APC but not GSTP1. This differential effect was observed because the borderline cases for RAR $\beta$ 2 and APC could now be unambiguously assigned as Cancer, improving the sensitivity. There were no borderline cases for GSTP1 in this data set, however, which is why the sensitivity remained unchanged.

Table 8

Pru-Mu - Norm S100			
	GSTP1	RAR	APC
Sensitivity	20.00%	15.00%	20.00%
Specificity	90.00%	100.00%	90.00%
GSTP1/APC//RAR $\beta$ 2			
Sensitivity		40.00%	
Specificity		80.00%	

45

50

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## SEQUENCE LISTING

SEQUENCE LISTING

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10 <120> Detecting Prostate Cancer

15 <130> P050151EP

20 <150> US11/734763

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## Claims

50 1. A method of detecting prostate cancer comprising, obtaining a urine sample from a person, and determining the methylation status of only the GSTP1 gene and one or more controls in the urine sample no later than three days after said urine sample was obtained; wherein methylation that exceeds a pre-determined value is indicative of prostate cancer and methylation that does not exceed such pre-determined value is indicative of the absence of prostate cancer.

55 2. A method of detecting prostate cancer comprising, obtaining a urine sample from a person, determining the methylation status of only the S 100 gene and only one more genes selected from the group consisting of the GSTP1

gene, APC, and RAR $\beta$ 2 and one or more controls in the urine sample no later than three days after said urine sample was obtained; wherein if the Ct value of the methylation of the GSTP1, APC, or RAR $\beta$ 2 gene less than that of the S 100 gene exceeds a pre-determined value it is indicative of prostate cancer and methylation that does not exceed such pre-determined value is indicative of the absence of prostate cancer.

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3. The method according to claim 1 or 2 further comprising measuring the presence of a reference Marker and wherein prior to the collection of said urine sample, the person is subjected to prostatic massage for about 20 seconds.
4. A method of detecting prostate cancer comprising, obtaining a urine sample from a patient, determining the methylation status of only the GSTP1 gene, the RAR $\beta$ 1, the APC gene and one or more controls in the urine sample; wherein methylation that exceeds a pre-determined value is indicative of prostate cancer and methylation that does not exceed such pre-determined value is indicative of the absence of prostate cancer wherein said method is conducted in a single vessel that is left unopened during the conduct of the method.
- 10 5. A method of detecting prostate cancer comprising, obtaining a urine sample from a patient with an abnormal DRE, determining the methylation status of only the GSTP1 gene, the RAR $\beta$ 1, the APC gene and one or more controls in the urine sample; wherein methylation that exceeds a pre-determined value is indicative of prostate cancer and methylation that does not exceed such pre-determined value is indicative of the absence of prostate cancer wherein said method is conducted in a single vessel that is left unopened during the conduct of the method.
- 15 6. A method of detecting prostate cancer comprising, obtaining a urine sample from a patient with an abnormal DRE, determining the methylation status of only the GSTP1 gene and the RAR $\beta$ 1 and one or more controls in the urine sample; wherein methylation that exceeds a pre-determined value is indicative of prostate cancer and methylation that does not exceed such pre-determined value is indicative of the absence of prostate cancer wherein said method is conducted in a single vessel that is left unopened during the conduct of the method.
- 20 7. The method according to claim 4 or 5 or 6 further comprising measuring the presence of a reference Marker.
8. The method of claim 4 or 5 or 6 wherein prior to the collection of said urine sample, the person is subjected to prostatic massage for about 20 seconds.
- 25 9. The method of claim 1, 2, 4, 5 or 6 further comprising the steps of determining the PSA level of said person and conducting said method only on people having a PSA level between 2.5 and 4 ng/ml.
- 10 10. The method of claim 1, 2, 4, 5 or 6 wherein the methylation status of the genes is determined using nested PCR wherein a first round of PCR is conducted followed by a subsequent round of PCR wherein primers and probes for conducting said subsequent PCR are directed to sequences within the sequences amplified in the first round of PCR and wherein the urine samples are spun down to form sediments prior to conducting the first round of PCR.
- 30 11. A kit for conducting an assay to detect prostate cancer, comprising: nucleic acid amplification and detection reagents for detecting the presence of genes consisting essentially of GSTP1 and one or more control genes and instructions that direct its use in patients in having measured PSA levels of 2.5-4 ng/ml.
12. A kit for conducting an assay to detect prostate cancer, comprising: nucleic acid amplification and detection reagents for detecting the presence of genes consisting essentially of GSTP1, RAR $\beta$ 1 and one or more control genes and instructions that direct its use in patients in having measured PSA levels of 2.5-4 ng/ml.
- 35 13. A kit for conducting an assay to detect prostate cancer, comprising: nucleic acid amplification and detection reagents for detecting the presence of genes consisting essentially of GSTP, RAR $\beta$ 1, APC and one or more control genes and instructions that direct its use in patients in having measured PSA levels of 2.5-4 ng/ml.
14. A kit for conducting an assay to detect prostate cancer, comprising: nucleic acid amplification and detection reagents for detecting the presence of the S 100A2 gene and one more genes selected from the group consisting of GSTP, RAR  $\beta$ 1, and APC, and one or more control genes.



DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (IPC)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	ROUPRÊT MORGAN ET AL: "Molecular detection of localized prostate cancer using quantitative methylation-specific PCR on urinary cells obtained following prostate massage." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH 15 MAR 2007, vol. 13, no. 6, 15 March 2007 (2007-03-15), pages 1720-1725, XP002485530 ISSN: 1078-0432 * abstract * * page 1721, column 2, paragraph 2 * * table 3 *	1-10	INV. G01N33/574
Y	-----	11-14	
X	HOQUE MOHAMMAD OBAIDUL ET AL: "Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects." JOURNAL OF CLINICAL ONCOLOGY : OFFICIAL JOURNAL OF THE AMERICAN SOCIETY OF CLINICAL ONCOLOGY 20 SEP 2005, vol. 23, no. 27, 20 September 2005 (2005-09-20), pages 6569-6575, XP002485531 ISSN: 0732-183X * abstract * * page 6570, column 2, paragraph 1; figure 1 *	1-10	TECHNICAL FIELDS SEARCHED (IPC)
Y	-----	11-14	G01N
X	WO 03/044232 A (UNIV JOHNS HOPKINS MED [US]; SIDRANSKY DAVID [US]) 30 May 2003 (2003-05-30) * abstract *	1	
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	-/-		
The present search report has been drawn up for all claims			
1	Place of search	Date of completion of the search	Examiner
	Munich	24 June 2008	Bigot-Maucher, Cora
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			



DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (IPC)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	BASTIAN PATRICK J ET AL: "Molecular biomarker in prostate cancer: the role of CpG island hypermethylation." EUROPEAN UROLOGY DEC 2004, vol. 46, no. 6, December 2004 (2004-12), pages 698-708, XP004645485 ISSN: 0302-2838 * abstract * * page 701, column 2, paragraph 1 * * page 704, column 2, paragraph 2; figure 3 * -----	2-10, 12-14	
X	YEGNASUBRAMANIAN S ET AL: "Hypermethylation of CpG islands in primary and metastatic human prostate cancer" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 64, no. 6, 15 March 2004 (2004-03-15), pages 1975-1986, XP002338830 ISSN: 0008-5472 * abstract; table 3 * * page 1980, column 1, paragraph 1 * -----	4-10, 12-14	TECHNICAL FIELDS SEARCHED (IPC)
X	JERÓNIMO CARMEN ET AL: "A quantitative promoter methylation profile of prostate cancer." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH 15 DEC 2004, vol. 10, no. 24, 15 December 2004 (2004-12-15), pages 8472-8478, XP002485532 ISSN: 1078-0432 * abstract * ----- -----	2,4-10, 14 -/-	
1	The present search report has been drawn up for all claims		
	Place of search	Date of completion of the search	Examiner
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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (IPC)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	BERNARDINI S ET AL: "Hypermethylation of the CPG islands in the promoter region of the GSTP1 gene in prostate cancer: a useful diagnostic and prognostic marker?" CLINICA CHIMICA ACTA, AMSTERDAM, NL, vol. 350, no. 1-2, 1 December 2004 (2004-12-01), pages 181-188, XP004629826 ISSN: 0009-8981 * abstract * * page 184, column 2, paragraph 3; figure 1 *	11-14	
X	----- CATALONA WILLIAM J ET AL: "Viewpoint: expanding prostate cancer screening." ANNALS OF INTERNAL MEDICINE 21 MAR 2006, vol. 144, no. 6, 21 March 2006 (2006-03-21), pages 441-443, XP002485533 ISSN: 1539-3704 * abstract * * page 441, column 1, last paragraph *	11-14	TECHNICAL FIELDS SEARCHED (IPC)
T	----- VENER TATIANA ET AL: "Development of a multiplexed urine assay for prostate cancer diagnosis" CLINICAL CHEMISTRY, vol. 54, no. 5, May 2008 (2008-05), pages 874-882, XP001538183 ISSN: 0009-9147 * the whole document *	1-14	
	----- ----- -/-		
1	The present search report has been drawn up for all claims		
	Place of search	Date of completion of the search	Examiner
	Munich	24 June 2008	Bigot-Maucher, Cora
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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (IPC)						
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim							
T	<p>MEHROTRA JYOTI ET AL: "Quantitative, spatial resolution of the epigenetic field effect in prostate cancer." THE PROSTATE 1 FEB 2008, vol. 68, no. 2, 1 February 2008 (2008-02-01), pages 152-160, XP002485534 ISSN: 0270-4137 * the whole document *</p> <p>-----</p>	1-14							
			TECHNICAL FIELDS SEARCHED (IPC)						
1	<p>The present search report has been drawn up for all claims</p> <table border="1"> <tr> <td>Place of search</td> <td>Date of completion of the search</td> <td>Examiner</td> </tr> <tr> <td>Munich</td> <td>24 June 2008</td> <td>Bigot-Maucher, Cora</td> </tr> </table> <p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... &amp; : member of the same patent family, corresponding document</p>			Place of search	Date of completion of the search	Examiner	Munich	24 June 2008	Bigot-Maucher, Cora
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Munich	24 June 2008	Bigot-Maucher, Cora							

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ON EUROPEAN PATENT APPLICATION NO.**

EP 08 25 1398

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

24-06-2008

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 03044232	A 30-05-2003		AT 393240 T	15-05-2008
			AU 2002352745 A1	10-06-2003
			CA 2467455 A1	30-05-2003
			EP 1456413 A1	15-09-2004
			JP 2005509445 T	14-04-2005

## REFERENCES CITED IN THE DESCRIPTION

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

## Patent documents cited in the description

- US 6326145 B [0024]
- US 6270967 B, Whitcombe [0024]
- US 4458066 A [0033]
- US 4683195 A, Mullis [0042]

## Non-patent literature cited in the description

- **C. B. PICKETT et al.** *Annu. Rev. Biochem.*, 1989, vol. 58, 743 [0003]
- **B. COLES et al.** *CRC Crit. Rev. Biochem. Mol. Biol.*, 1990, vol. 25, 47 [0003]
- **T. H. RUSHMORE et al.** *J. Biol. Chem.*, 1993, vol. 268, 11475 [0003]
- **B. MANNERVIK et al.** *Biochem. J.*, 1992, vol. 282, 305 [0003]
- **BEAUCAGE.** *Tetrahedron Letters*, 1981, vol. 22, 1859-1862 [0033]
- *CSH-Quantitative Biology*, 1978, vol. 43, 63 [0035]
- **C. RADDING.** *Ann. Rev. Genetics*, 1982, vol. 16, 405-437 [0035]